

10/516616

PCT/IL 03/00463

Rec'd PCT/PTO 03 DEC 2004



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מספר:  
Number  
**150016**

תאריך:  
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**04-06-2002**

חוקדם/נדחה:  
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אני, (שם המבקש, מענו ולגבי גוף מאוגדת מקום התאגדותו)  
I, (Name and address of applicant, and in case of body corporate-place of incorporation)

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Agents for imaging and diagnostic methods using them

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Application of Division		Appl. for Patent of Addition		Priority Claim		
מבקשת פטנט from application		לבקשה/לפטנט to Patent/Appl.		מספר/סימן Number/Mark	תאריך Date	מדינת האיגוד Convention Country
No.	מס'	No.	מס'			
Dated	מיום	Dated	מיום			
P.O.A.: General		כללי				
filed in case		P140111				
C. 137952		המען למסירת מסמכים בישראל Address for Service in Israel				
REINHOLD COHN AND PARTNERS		ריינהולד כהן ושותפיו				
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**גורמים להדמיה ושיטות איבחון המשתמשים בהם**

**Agents for imaging and diagnostic methods using them**

**NST Neuro Survival Technologies Ltd.**

**נסט נירוסורויול טכנולוגיס בע"מ**

**C. 137952**

## AGENTS FOR IMAGING AND DIAGNOSTIC METHODS USING THEM

### FIELD OF THE INVENTION

The present invention relates to novel compounds useful as imaging agents, and diagnostic methods using them for detecting a disease process, for monitoring the progression of a disease and/or for monitoring of the effect of treatment.

5

### LIST OF REFERENCES

The following references are considered to be pertinent for the purpose of understanding the background of the present invention:

- Beyers, E.M., *et al.*, *Biochim. Biophys. Acta*, 1439:317-330, 1999;
- 10 Bombeli, T., *et al.*, *Blood*, 89:2429-2442, 1997;
- Bratton, D.L., *et al.*, *J. Biol. Chem.*, 272:26159-26165, 1997;
- Bursch, W., *et al.*, *Trends Pharmacol. Sci.*, 13:245-251, 1992;
- Kockx M.M., *et al.*, *Cardiovasc. Res.*, 45:736-746, 2000;
- Mallat, Z., *et al.*, *Circulation*, 96:424-428, 1997;
- 15 Martin, S., *et al.*, *J. Exp. Med.*, 182:1545-1556, 1995;
- Sims, P.J., *et al.*, *Thromb. Haemost.*, 86:266-275, 2001;
- Stary, H.C., *et al.*, *Circulation*, 92:1355-1374, 1995;
- Van den Eijnde, S.M., *et al.*, *Cell Death Diff.*, 4:311-316, 1997.

The above references will be acknowledged in the text below by indicating in brackets, from the above list, the name of the first author and the year of publication.

## BACKGROUND OF THE INVENTION

5 Cell membranes of intact eukaryotic cells are characterized by a highly organized structure. This high level of organization is determined, among others, by the molecular structure of the specific lipids constituting the membranes; the ratio between the various lipid species from which the membrane is composed; the distribution of the phospholipids between the outer and inner leaflets of the  
10 membranes; and by the protein components of the membrane.

While maintenance of the high level of membrane organization is fundamental to normal cell physiology, substantial perturbations and alterations of the normal organization of membrane (PNOM) occur in numerous physiological and pathological conditions, and are characterizing a plurality of diseases (Martin,  
15 S., *et al.*, 1995). Such alterations and perturbations may be evident both at the morphological level (membrane blebbing observed in cells undergoing apoptosis) and at the molecular level. The scope of perturbations accompanying either cell activation, cell disease or cell death is not fully elucidated. They include, among others, scrambling and redistribution of the membrane phospholipids, with  
20 movement to the cell surface of aminophospholipids, mainly phosphatidylserine (PS) and phosphatidylethanolamine (PE), which are normally restricted almost entirely to the inner leaflet of the membrane bilayer, and movement of sphingomyelin and phosphatidylcholine from the outer leaflet to the inner leaflet of the membrane (Sims, P.J., *et al.*, 2001). This redistribution is referred herein as loss  
25 of cell membrane lipid asymmetry (CMLA). These alterations play an indispensable role in making the cell surface a catalytic platform for the assembly of several clotting factor complexes, such as tenase and prothrombinase complexes (Bever, E.M., *et al.*, 1999). Thus, platelets undergo PNOM upon activation, and these alterations constitute an important factor in normal blood coagulation, as well

as in the initiation and/or propagation of abnormal, excessive blood clotting in numerous disorders. These disorders include, among others, arterial or venous thrombosis or thrombo-embolism [e.g., cerebral stroke, myocardial infarction, deep vein thrombosis (DVT), disseminated intravascular coagulation (DIC), thrombotic  
5 thrombocytopenic purpura, etc.]; unstable atherosclerotic plaques, sickle cell disease; beta-thalassemia; anti-phospholipid antibody syndrome; among others in systemic lupus erythematosus (SLE); disorders associated with shedding of membrane microparticles, e.g., neurological dysfunction in association with cardiopulmonary bypass.

10 Apoptosis is another major situation in which alterations/perturbations of cellular membranes take place (Bratton, D.L., *et al.*, 1997). Apoptosis is an intrinsic program of cell self- destruction or "*suicide*", which is inherent in every eukaryotic cell. In response to a triggering stimulus, cells undergo a highly characteristic cascade of events of cell shrinkage, blebbing of cell membranes, chromatin  
15 condensation and fragmentation, culminating in cell conversion to clusters of membrane-bound particles (apoptotic bodies), which are thereafter engulfed by macrophages (Bursch, W., *et al.*, 1992). PNOM is a universal phenomenon in apoptosis, it occurs early in the apoptotic cascade, probably at the point of cell commitment to the death process, and has also been shown to be an important  
20 factor in the recognition and removal of apoptotic cells by macrophages (Van den Eijnde, S.M., *et al.*, 1997).

A strong correlation has been recently drawn between PNOM and potent procoagulant activity of apoptotic cells (Bombeli, T., *et al.*, 1997). PNOM in apoptotic endothelial cells, such as in atherosclerotic plaques (Mallat, Z., *et al.*,  
25 1997), probably plays an important role in the pathogenesis of thrombotic vascular disorders. PNOM is also a feature of inflammatory cells (i.e., lymphocytes, macrophages), activated by various triggers.

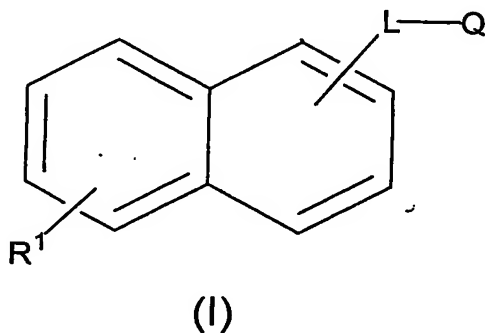
Since apoptosis, thrombosis or inflammation have an important role in the majority of medical disorders, it is desirable to have tools for detection of these  
30 biological processes. Compounds for selective binding to PNOM membranes,

potentially also performing subsequent entry into these cells having such PNOM membranes, may therefore serve as an important tool for detecting and targeting of cells undergoing activation, damage or death process, especially by apoptosis. In the clinical context, detection of binding to said membranes may be useful in the diagnosis of disease processes, in monitoring course or progression of a disease, or in monitoring the effect of various therapeutic approaches utilized to alter disease course.

### SUMMARY OF THE INVENTION

It is an object of the present invention to provide new compounds and their uses as imaging agents for detecting a disease process, for monitoring the progression of a disease process, and / or for monitoring of the results of therapy.

According to one aspect, the present invention provides new compounds having the following formula  $E_i$ , wherein  $i$  is 1 or 2 and  $E$  is a group having the formula (I):



including pharmaceutically acceptable salts, metal chelates and hydrates of the structure of formula (I); wherein

$Q$  is a marker for imaging, or a metal chelator, the marker for imaging being selected from the group comprising a fluorescent label, a radio-label, a marker for X-ray, a marker for MRI, a marker for PET scan, or a label capable of undergoing an enzymatic reaction that produces a detectable color;

L is a linking group having the formula  $D-U-B-Y^1-A-Y^2$ , wherein D is selected from the group consisting of O, S, SO, SO<sub>2</sub>, SO<sub>2</sub>NH, NHSO<sub>2</sub>, NH, PO, PO<sub>2</sub>, PO(NH)<sub>2</sub>, NHPO<sub>2</sub>, CO, CO<sub>2</sub>, NHCO, CONH, SO<sub>2</sub>NHCHCO<sub>2</sub>, and SO<sub>2</sub>NHCO;

5 U is selected from optionally substituted C<sub>1</sub>-C<sub>6</sub> alkylene, C<sub>2</sub>-C<sub>6</sub> alkenylene, C<sub>3</sub>-C<sub>6</sub> branched alkylene, and C<sub>3</sub>-C<sub>6</sub> branched alkenylene;

Y<sup>1</sup> and Y<sup>2</sup> may be the same or different, and are each independently selected from null or U;

B is null or is selected from O, S, NH, C=O, CH<sub>2</sub>-OH, (CH<sub>2</sub>)<sub>2</sub>-OH,  
10 CH<sub>2</sub>-NH<sub>2</sub>, (CH<sub>2</sub>)<sub>2</sub>-NH<sub>2</sub>, aryl, heteroaryl, and combinations thereof;

A groups may be the same or different, and are charged moieties at pH of about 7; said charged moieties being either positively-charged, negatively-charged or in zwitterion form;

where when  $i = 2$ , the compound of formula (I) is in dimeric form (E-E),  
15 said dimeric form having:

a single Q moiety;

a single A group; and

in the case that Q is a charged group at pH of about 7, said A group of said E-E dimeric form being as defined above or null;

20 R<sup>1</sup> is WR<sup>2</sup><sub>b</sub>, where W is null or is selected from N, O, S and C;

R<sup>2</sup> represents hydrogen or a C<sub>1</sub>-C<sub>6</sub> alkyl; R<sup>2</sup> moieties may be either the same or different; and

b is 1, 2 or 3.

In a preferred embodiment, in the above formula (I) Q is a metal chelator.  
25 Advantageously, Q forms metal chelates with Technetium, Gallium or Rhenium isotopes.

Preferably, U is an optionally substituted C<sub>1</sub>-C<sub>5</sub> alkylene group; R<sup>1</sup> is NR<sup>2</sup><sub>2</sub>, wherein each R<sup>2</sup> group is selected independently from hydrogen and C<sub>1</sub>-C<sub>4</sub> alkyl;

A is a charged group at pH of about 7, said charged group being either  
30 positively-charged, negatively-charged or in zwitterion form and comprises at least

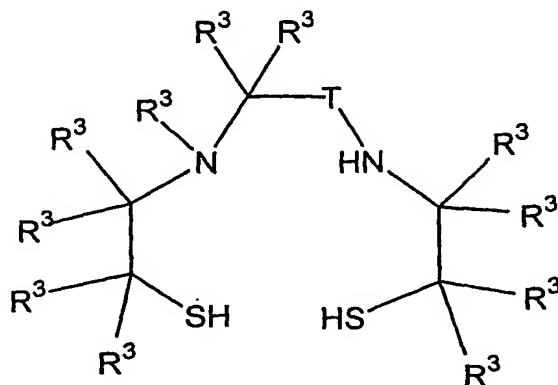
one amino group and at least one acidic group; the acidic group being selected from carboxylic, phosphoric, phosphatic, sulfonic and sulfuric acid; the amino group may also be a quaternary ammonium ion; and

Q is a metal chelator, where the chelation to a metal is accomplished through a combination of nitrogen, sulfur and/or oxygen atoms comprised by Q. Preferably, said chelation is accomplished by three nitrogen atoms and a sulfur atom or two nitrogen atoms and two sulfur atoms or a nitrogen atom and three sulfur atoms. Examples of such metal chelators are chelators comprising diaminedithiols, monoamine-monoamide-bisthiols (MAMA), triamide-monothiols, and monoamine-diamide-monothiols.

In another preferred embodiment, the B group is other than null; and the D-U moiety has the formula  $-\text{SO}_2\text{NH}-(\text{CH}_2)_n$ , wherein n is an integer between 1-3 and Q, A and Y are as defined above. Preferably, n is 2.

In another preferred embodiment B is null, A is a positively-charged group at pH of about 7,  $\text{Y}^1$  is null,  $\text{Y}^2$  and Q are as defined above, and D-U is of the formula  $-\text{SO}_2\text{NH}-(\text{CH}_2)_n$ , wherein n is an integer, independently selected among 1-3. Preferably, n is 2.

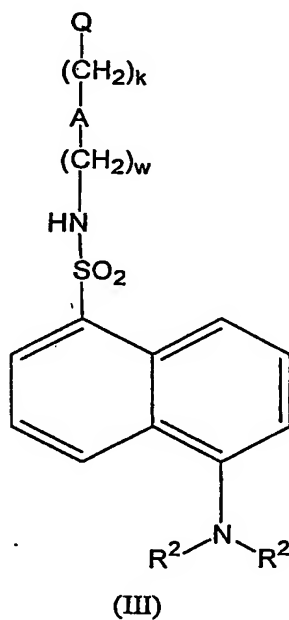
Preferably, Q is a metal chelator of the following formula (II):



(II)

wherein T is CH<sub>2</sub> or C=O; R<sup>3</sup> groups may be the same or different, and are each independently selected from hydrogen, methyl, ethyl, COOH and CH<sub>2</sub>COOH; wherein the number of R<sup>3</sup> moieties comprising a carboxyl group ≤ 2; including pharmaceutically acceptable salts, and hydrates thereof; said metal chelator Q being  
5 bound to L via an R<sup>3</sup> group or a nitrogen atom of the chelator.

In yet another preferred embodiment, the new compounds of the invention have the following formula (III):

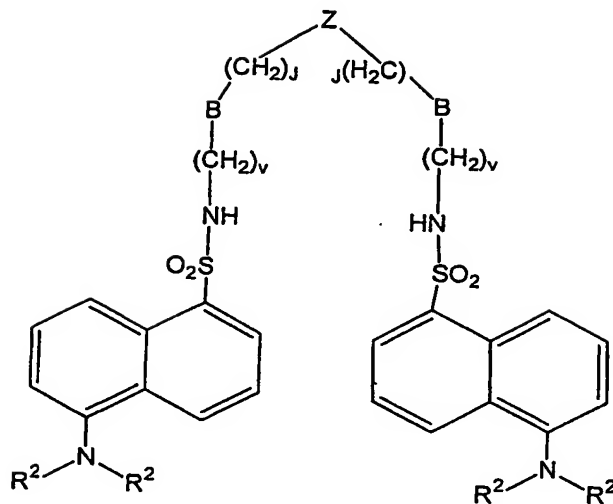


10

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula III;

wherein A and Q have the same meanings as above; and R<sup>2</sup> groups may be the same or different and are each independently selected from hydrogen and C<sub>1</sub>-C<sub>4</sub>  
15 alkyl and k and w are integers of 1-3. Preferably, R<sup>2</sup> is methyl.

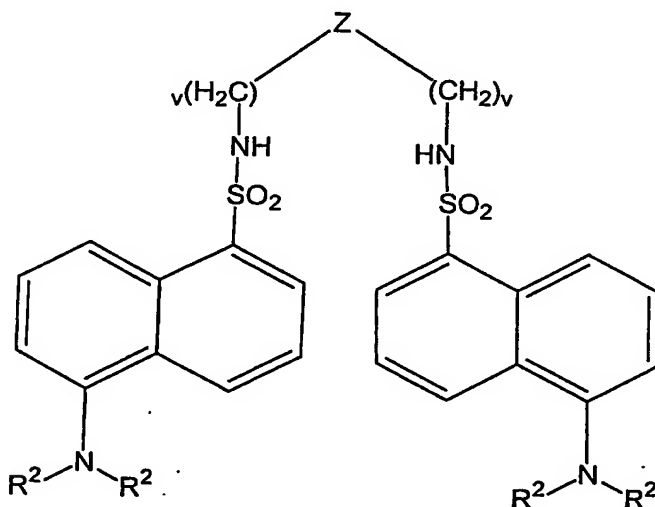
In another preferred embodiment, the compounds of the present invention have the following formula (IV):



(IV)

wherein R<sup>2</sup> has the same meaning as above; Z is selected from Q and A-Y<sup>2</sup>-Q as defined above; and v and j are each integer of 0-3; including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula IV.  
 5 Preferably, R<sup>2</sup> is methyl.

In yet another preferred embodiment, the compounds of the present invention have the following formula (V):

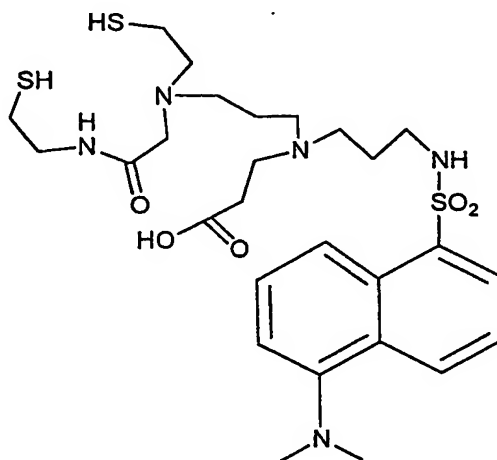


(V)

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula V;

wherein  $R^2$  has the same meaning as above; Z is selected from Q, A- $Y^2$ -Q, B- $Y^1$ -A-Q, and B- $Y^1$ -Q as defined above; and v is an integer of 1-6; wherein in the case that Z is B- $Y^1$ -Q, Q is a charged group (at pH of about 7). Preferably,  $R^2$  is methyl and v is an integer of 1-3.

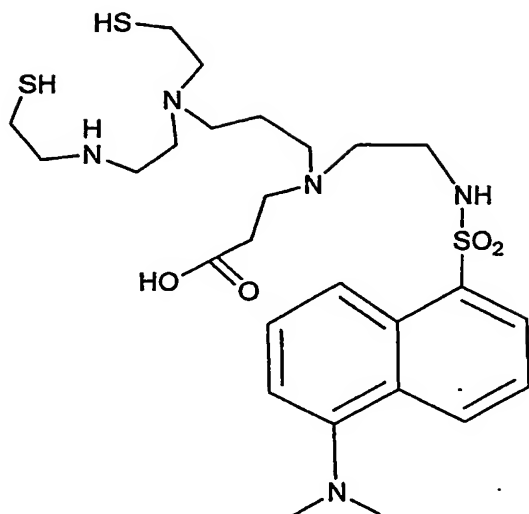
In a specific embodiment, the compound of the invention has the following formula VI and is designated NST 901A:



(VI)

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula VI. Preferably, said metal chelates are Technetium or Rhenium chelates.

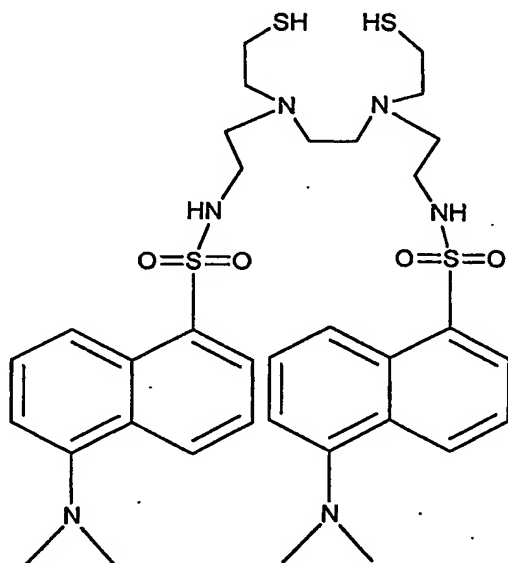
In another specific embodiment the compound of the invention has the following formula VII and is designated NST 901B:



(VII)

including pharmaceutically acceptable salts, hydrates and metal chelates of said compound of the formula VII. Preferably, said metal chelates are Technetium  
5 or Rhenium chelates.

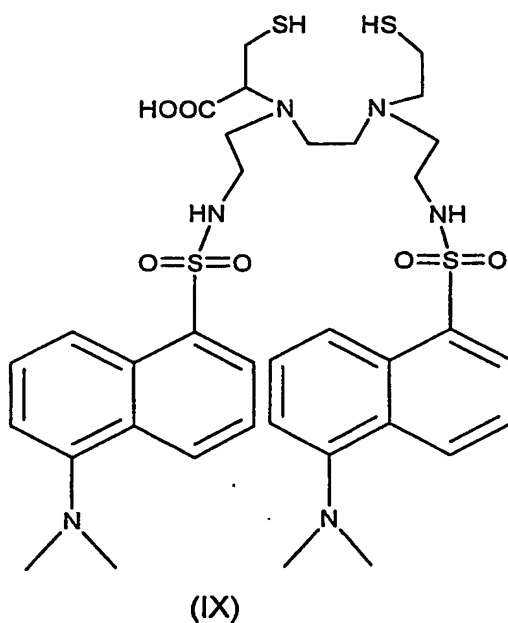
In another specific embodiment, the compound of the invention has the following formula VIII and is designated NST 902:



(VIII)

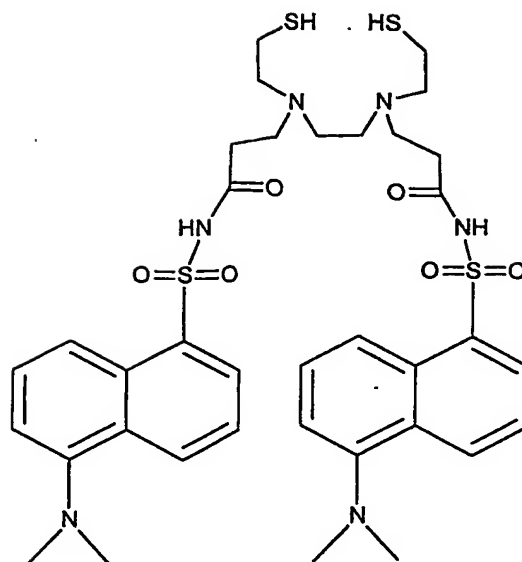
including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula VIII. Preferably, said metal chelates are Technetium or Rhenium chelates.

In another specific embodiment the compound has the following formula IX and is designated NST903:



including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula IX. Preferably, said metal chelates are Technetium or Rhenium chelates.

In another specific embodiment, the compound of the invention has the following formula X, and is designated NST 904:



(X)

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula X. Preferably, said metal chelates are Technetium or Rhenium chelates.

One desired property of the compounds of the present invention, is the selective binding of said compounds to membranes of cells, undergoing perturbation of their normal membrane organization (PNOM), with potential subsequent entry into said cells, while essentially not binding / entering to, or binding / entering to a much lesser degree to cells maintaining their normal organization. This property may be useful for the detection of cells or cell-derived particles, which contain PNOM membranes (PM), said cells being designated "PM cells", and said detection being designated the "detection aspect" of the invention. The term PNOM for the purpose of the present invention refers to a cell membrane featuring at least one of the following:

- (i) Scrambling of membrane phospholipids, with reduction of normal asymmetry of distribution of phospholipids between the inner and outer leaflets of the cell membrane;
- (ii) Exposure of aminophospholipids on the outer cell surface (mainly exposure of phosphatidylserine and phosphatidylethanolamine);
- (iii) Impairment of packing of membrane constituents;

(iv) Impairment of normal distribution of lipids within each membrane leaflet, such as formation of lateral domains, being either enriched or poor in a specific lipid membrane constituent, e.g., phosphatidylserine or cholesterol, respectively.

5 Therefore, the compounds of the invention may be used for the diagnosis of physiological disorders in which cells undergo PNOM as will be explained herein below.

Thus, according to another of its aspects, the present invention provides a novel diagnostic agent, comprising a compound  $E_i$  as defined above, and a metal  
10 chelated to said compound through a Q moiety thereof, wherein Q is a metal chelator, said metal being the source of a signal detectable by one or more imaging techniques [e.g., radio-isotope scan, magnetic resonance imaging (MRI)].

According to another aspect, the diagnostic agent is a compound of the Formula  $E_i$  as defined above, wherein Q is a radioisotope of a material other than a  
15 metal covalently linked to L, said radioisotope being the source of a signal detectable by imaging technique, and wherein Q and L are as defined above.

Optionally, the diagnostic agent is a compound of the Formula  $E_i$  as defined above, having fluorescent properties that may be detected by fluorescence techniques (e.g. a fluorescent microscope).

20 In another aspect thereof, the present invention provides a diagnostic composition comprising an active component that is a compound of the invention of the formula  $E_i$ , that have detectable properties of its own or is capable of chelating a detectable label such as a metal, together with a biologically acceptable carrier, for the detection of PM cells, in a sample of biological cells, *in vitro*, *ex vivo*, *in vivo* or for clinical imaging. The active compound of the present invention  
25 is capable of selectively binding / entering PM cells present in the assayed sample. Subsequently, said binding may be identified by any means known in the art.

According to another aspect, the present invention provides a diagnostic kit for the administration of a diagnostic composition or a diagnostic agent to a subject  
30 in order to diagnose a physiological disorder. Such diagnostic kit comprises one or

more vials containing a sterile formulation comprised of a predetermined amount of a diagnostic composition or diagnostic agent of the invention and optionally other components, such as stabilization aids, solubilization aids or bacteriostats. The one or more vials that contain all or part of the formulation can independently be in the  
5 form of a sterile solution or a lyophilized solid.

In a preferred embodiment, the diagnostic composition is a diagnostic radiocomposition, for radioimaging by standard radioimaging techniques, such as single photon emission computed tomography (SPECT), the metal being a radioisotope of the following metal atoms: Tc, In, Cu, Ga, Xe, Tl and Re, preferably Tc and Re; or the covalently linked radiolabel is selected from:  $^{123}\text{I}$  and  $^{131}\text{I}$ . For example, a preferred embodiment is a compound of the invention, radiolabeled with  $^{99\text{m}}\text{Tc}$ , for detection by SPECT.  
10

In another preferred embodiment, the diagnostic composition is a diagnostic radiocomposition for Positron Emission Tomography (PET) scan, comprising a  
15 covalently linked radiolabel (the Q moiety), selected from the group  $^{18}\text{F}$ ,  $^{15}\text{O}$ ,  $^{18}\text{O}$ ,  $^{11}\text{C}$ ,  $^{13}\text{C}$ ,  $^{124}\text{I}$ ,  $^{13}\text{N}$  and  $^{75}\text{Br}$ .

In yet another preferred embodiment, the diagnostic composition is a MRI contrast composition, the metal being a paramagnetic metal ion selected from: Gd(III), Fe(III) or Mn(II).

20 In yet another preferred embodiment, the diagnostic composition is an X-ray or computerized tomography (CT) contrast composition, comprising a contrast agent such as Ba, Cs, Re, Rh, Ag, Ir or iodine.

The term "*disease characterized by PM*" refers to a disease of which one of its manifestations is PM cells, occurring in tissues inflicted by the disease. This is  
25 not meant to indicate that this perturbation of normal membrane structure of these cells is necessarily the cause, or the sole effect of the disease, but rather that it is one of its manifestations.

The compounds and the diagnostic agents of the invention can be used for a detection of the following conditions:

- 30 1) apoptotic cells and apoptotic bodies;

- 2) damaged cells and cells undergoing non-apoptotic modes of cell death;
- 3) activated platelets;
- 4) activated inflammatory cells, such as activated white blood cells or tissue macrophages.

5        Therefore, the compounds and the agents of the invention can be useful in the diagnosis of a wide variety of biological conditions, in which the above cells and cell-derived particles have a role. These include physiological conditions such as tissue development or aging, and various pathological conditions.

10        According to the *detection aspect* of the invention, the compounds of the invention may be used for the diagnosis of medical disorders in which cells undergo PNOM, monitoring the progression of said medical disorders or monitoring the effects of treatments administered to patients suffering from said diseases. Examples of such medical disorders are as follows:

15        *Diseases which are characterized by occurrence of excessive apoptosis,* such as degenerative disorders, neurodegenerative disorders (e.g., Parkinson's disease, Alzheimer's disease, Huntington chorea), AIDS, myelodysplastic syndromes, ischemic or toxic insults, graft cell loss during transplant rejection; tumors, and especially highly malignant / aggressive tumors, are also often characterized by enhanced apoptosis, in addition to the excessive tissue  
20 proliferation.

25        *Diseases manifested by excessive blood clotting,* wherein PNOM occur during platelet activation, and / or during activation of or damage to other cellular elements (e.g., endothelial cells). These diseases include, among others, arterial or venous thrombosis, thrombo-embolism, e.g., myocardial infarction, cerebral stroke, deep vein thrombosis, disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), sickle cell diseases, thalassemia, antiphospholipid antibody syndrome, systemic lupus erythematosus.

30        *Inflammatory disorders, and / or diseases associated with immune-mediated etiology or pathogenesis,* such as auto-immune disorders such as antiphospholipid antibody syndrome, systemic lupus erythematosus, connective

tissue disorders such as rheumatoid arthritis, scleroderma; thyroiditis; dermatological disorders such as pemphigus or erythema nodosum; autoimmune hematological disorders; autoimmune neurological disorders such as myasthenia gravis; multiple sclerosis; inflammatory bowel disorders such as ulcerative colitis;  
5 vasculitis.

*Atherosclerotic plaques*, and especially plaques that are unstable, vulnerable and prone to rupture, are also characterized by PM cells, comprising apoptotic macrophages, apoptotic smooth muscle cells, apoptotic endothelial cells, activated platelets and activated inflammatory cells. In addition, the compounds of the  
10 present invention are capable of binding to the extracellular lipid, characterizing said atherosclerotic plaques, and thus allow imaging of said lipid core of the atherosclerotic plaque.

The detection of these pathological conditions, disorders or diseases via detection of the PM cells may be an aim by itself, simply for diagnosis of the  
15 presence of a disease condition in a specific individual.

Said detection may also be carried out in a person already known to have the disease for the purpose of evaluating the disease severity and / or in order to monitor response to various therapeutic modalities. An example for such monitoring is evaluation of response to anticancer therapy. Since most anti-tumor  
20 treatments, chemotherapy or radiotherapy exert their effect by induction of cell death, and particularly apoptosis, detection by the agents of the invention of therapy-induced apoptosis of tumor cells may substantially assist in the proper evaluation of the efficacy of said treatments.

Moreover, said detection may be used to monitor adverse effects of  
25 anti-cancer treatments. A large part of such adverse effects are due to untoward treatment-induced apoptosis of normal, yet sensitive cells, such as various types of epithelial cells or cells of the bone marrow hematopoietic system. Detection by the compounds of the invention of such apoptosis may allow early detection of this untoward tissue damage and better optimization of the treatment protocol.

In addition, said detection may aim at characterization of intrinsic apoptotic load within a tumor, characterization of the level of aggressiveness of a tumor, and detection of metastases.

Similarly, the compounds of the current invention may be useful in  
5 monitoring graft survival after organ transplantation, since apoptosis, potentially detectable by the compounds of the invention, plays a major role in cell loss during graft rejection. Early diagnosis of such rejection is of major clinical importance, and is currently being achieved by recurrent invasive and potentially dangerous tissue biopsies. The method of the invention may be useful as a non-invasive  
10 method for detecting cell death within the graft, and its imaging.

In addition, said detection may be useful for monitoring response to cyto-protective treatments, administered to inhibit cell death in disorders such as degenerative disorders or various ischemic and toxic insults. Thus the compounds and method of the present invention may aid in the screening and development of  
15 drugs which are capable of inhibiting cell loss in various diseases (for example those recited above) by enabling a measure of evaluation of cell death.

Said detection may be also useful for detection of atherosclerotic plaques, since the destabilization of such plaques, rendering them vulnerable, prone to rupture, thrombosis and embolization, is characterized by participation of several  
20 elements, of which have in common perturbed membranes: (i). apoptotic cells: the unstable plaque is characterized by apoptotic macrophages, apoptotic smooth muscle cells, and apoptotic endothelial cells (ii). activated platelets (iii). activated inflammatory cells. A lipid core, comprising of extracellular accumulation of lipids is also one of the hallmarks of atherosclerotic plaques undergoing destabilization  
25 (Kockx M.M., *et al.*, 2000; Stary, H.C., *et al.*, 1995). The compounds of the invention may be useful, due to their hydrophobic aromatic component, also for the binding to and identification of such lipid core of the atherosclerotic plaque.

The detection may also take place for basic research purposes in the study of apoptosis in tissue culture and animal models, and may help in determining the role  
30 of apoptosis not only in disease states, but also in normal development and

homeostasis of various tissues, such as in the development of the central nervous system during embryogenesis, as well as during situations such as normal aging.

In accordance with this approach, the present invention further concerns a method for the detection of PM cells, the method comprising:

- 5 (i) contacting the cell sample with a diagnostic agent of the invention under conditions enabling binding / accumulation of said agent in cells;
- (ii) detecting compound in said cells; the presence of a significant amount of compound in cells indicating the presence of PNOM in said cells.

10 The method of the present invention may be used for the diagnosis of a disease characterized by the occurrence of PNOM, for example, any one of the diseases indicated above.

The method of the present invention may also be used for monitoring the effects of various therapeutic modalities for said diseases or medical conditions, or  
15 alternatively for basic science research purposes as explained above.

The composition of the invention may be administered by any of the known routes, *inter alia*, oral, intravenous, intraperitoneal, intramuscular, subcutaneous, sublingual, intragastric, intraocular, intranasal or topical administration. The carrier should be selected in accordance with the desired mode of administration, and  
20 include any known components, e.g. solvents; emulgators, excipients, talc; flavors; colors, etc. The pharmaceutical composition may also comprise, if desired, also other pharmaceutically-active compounds which are used to treat disease, eliminate side effects or augment the activity of the active component.

The present invention further provides a novel method for the detection of  
25 physiological disorders characterized by the presence of PM cells, and / or diseases in which PM cells have an etiological or a pathogenetic role, such method comprising:

- (1) administering a diagnostic composition of the present invention to a patient; and

- (2) imaging of the patient using an appropriate imaging technique, known to those of art.

In a preferred embodiment, the present invention provides a novel method for the detection of physiological disorders characterized by the presence of PM cells, and / or diseases in which PM cells have an etiological or a pathogenetic role, such method comprising:

- (1) administering a radiocomposition of the invention to a patient; and
- (2) imaging of the patient using radioimaging techniques known to those of the art, such as single photon emission tomography (SPECT) in the case of a radiocomposition comprising  $^{99m}\text{Tc}$ , or positron emission tomography (PET) in the case of a radiocomposition comprising  $^{18}\text{F}$ .

In another preferred embodiment, the present invention provides a novel method for the detection of physiological disorders characterized by the presence of PM cells, and / or diseases in which PM cells have an etiological or a pathogenetic role, such method comprising:

- (1) administering a MRI contrast composition of the invention to a patient; and
- (2) imaging the patient using magnetic resonance imaging techniques, known to those of the art.

In yet another preferred embodiment, the present invention provides a novel method for the detection of physiological disorders characterized by the presence of PM cells, and / or diseases in which PM cells have an etiological or a pathogenetic role, such method comprising:

- (1) administering a X-ray contrast composition of the present invention to a patient; and
- (2) imaging the patient using X-ray or computed tomography (CT) techniques, known to those of the art.

In yet another preferred embodiment, the present invention provides a novel method for the detection of physiological disorders characterized by the presence of

PM cells, and / or diseases in which PM cells have an etiological or a pathogenetic role, such method comprising:

- (1) administering a composition of the present invention to a patient; wherein the diagnostic agent is a fluorescence-emitting moiety; and
- 5 (2) imaging the patient using fluorescence techniques known to those of the art (e.g. a fluorescent microscope).

### BRIEF DESCRIPTION OF THE DRAWINGS

10 In order to understand the invention and to see how it may be carried-out in practice, a preferred embodiment will now be described, in which detection of binding / entering of compounds of the present invention into / to cells undergoing PNOM due to apoptosis was evaluated. Selective binding of Re-NST901A (i.e, NST901A in complex oxo-rhenium) is presented. Binding was measured by  
15 monitoring of the intensity of the intrinsic fluorescence of the compounds by fluorescent microscopy analysis. Said preferred embodiment will now be described by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 shows fluorescent microscopy demonstrating the selective binding  
20 of Re-NST901A to apoptotic cells, in an aged culture of HeLa cells;

Fig. 2 shows detection of apoptosis of small intestine epithelial cells, induced in mice by systemic administration of chemotherapy; said detection being performed by Re-NST901A *in vivo*.

### 25 DETAILED EXPLANATION OF FIGURES:

Fig. 1: Fluorescent microscopy demonstrating the selective binding of Re-NST901A to apoptotic cells, in an aged culture of HeLa cells;

HeLa cells were allowed to age in culture for 96 hours without exchange of  
30 the culture medium. Said procedure is known to induce apoptosis in a percentage of

the cells. Cells were then incubated with Re-NST901A (250  $\mu$ M) for 10 minutes, and were visualized using a fluorescent microscope (excitation wavelength of 360nm, emission at 530nm). Selective uptake of Re-NST901A and intracellular accumulation within the fraction of cells undergoing apoptosis was observed, while  
5 intact non-apoptotic cells remained un-stained (magnification is X 200).

**Fig. 2: Detection of chemotherapy-induced apoptosis of small intestine epithelial cells *in vivo* by Re-NST901A:**

Balb/c mice were treated with a single dose of chemotherapy [Taxol (27  
10 mg/kg) + cyclophosphamide (300 mg/kg)]. Twenty-four hours later, the animals were injected intravenously with Re-NST901A (210 mg / kg). Animals were sacrificed two hours later, and the small intestine tissue was removed and subjected to fluorescent histopathological analysis. *A.* Hematoxinilin and eosin (H/E) staining, showing apoptotic epithelial cells within the intestinal cryptae,  
15 manifested by eosinophilic cytoplasm, and condensed and fragmented nuclei (several of these cells are marked by yellow arrows). *B.* Detection of said apoptotic cells by Re-NST901A, showing marked uptake and accumulation of the compound within the apoptotic cells.

**EXAMPLES:**

20 **Example 1: Synthesis of thiol-protected NST901A (Scheme 1):**

4-Methoxybenzyl chloride (15.6g) was reacted with 2-aminoethanethiol (7.7g) in methanol and sodium methoxide, to afford of 2-(4-methoxybezyl-sulfanyl)-ethylamine (1) (18.8g). One half of this material was taken in dichloromethane (cooling bath 0°C). Chloroacetyl chloride in the same solvent,  
25 was added slowly with stirring, followed by an equivalent of triethyl amine to produce 2-chloro N-[2-(4-methoxybenzyl-sulfanyl)-ethyl]acetamide (2) in a 94% yield. Compound 2 was reacted with an equivalent amount of 1 in refluxing acetonitrile for 6 hrs to provide N-[2-(4-methoxy-benzylsulfanyl)-ethyl]-

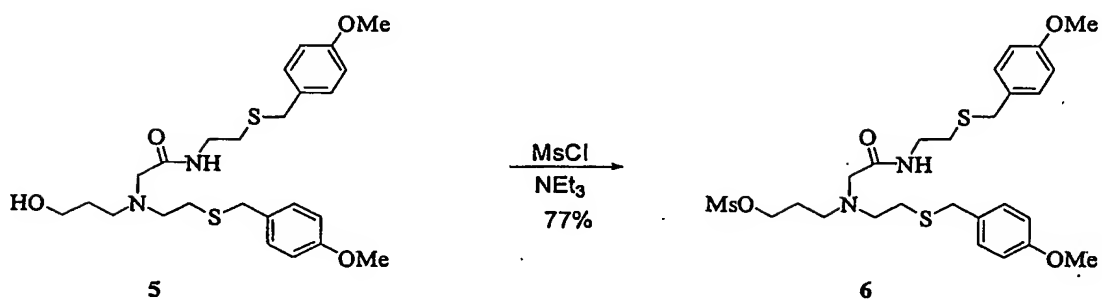
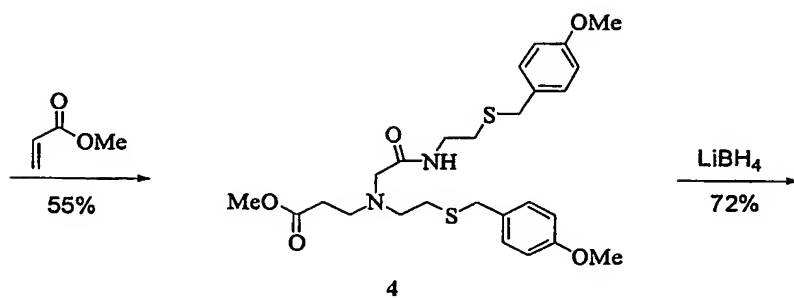
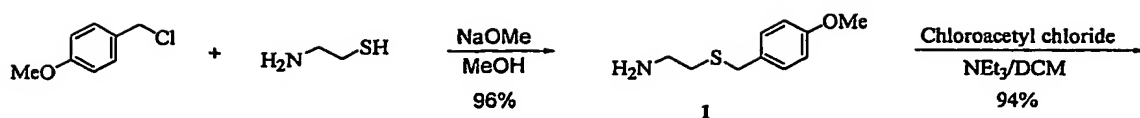
2-[2-(4-methoxy-benzylsulfanyl)-ethylamino]-acetamide (3) (40.4g) . 3 (10.8g) was then reacted with methyl acrylate in boiling methanol, for 10 hours, to produce 3-([2-(-methoxy-benzylsulfanyl)-ethyl]-{[2-(4-methoxy-benzylsulfanyl)-ethylcabamoyl]-methyl}-amino) propionic acid methyl ester (4) 7.15g isolated  
5 yield after column chromatography. This ester was reduced cleanly, with lithium borohydride to afford 3-([2-(-methoxy-benzylsulfanyl)-ethyl]-{[2-(4-methoxy-benzylsulfanyl)-ethylcabamoyl]-methyl}-amino)propionyl alcohol (5). The hydroxyl group was next converted to mesylate (6) using methanesulfonic anhydride and triethylamine in a 68% yield.

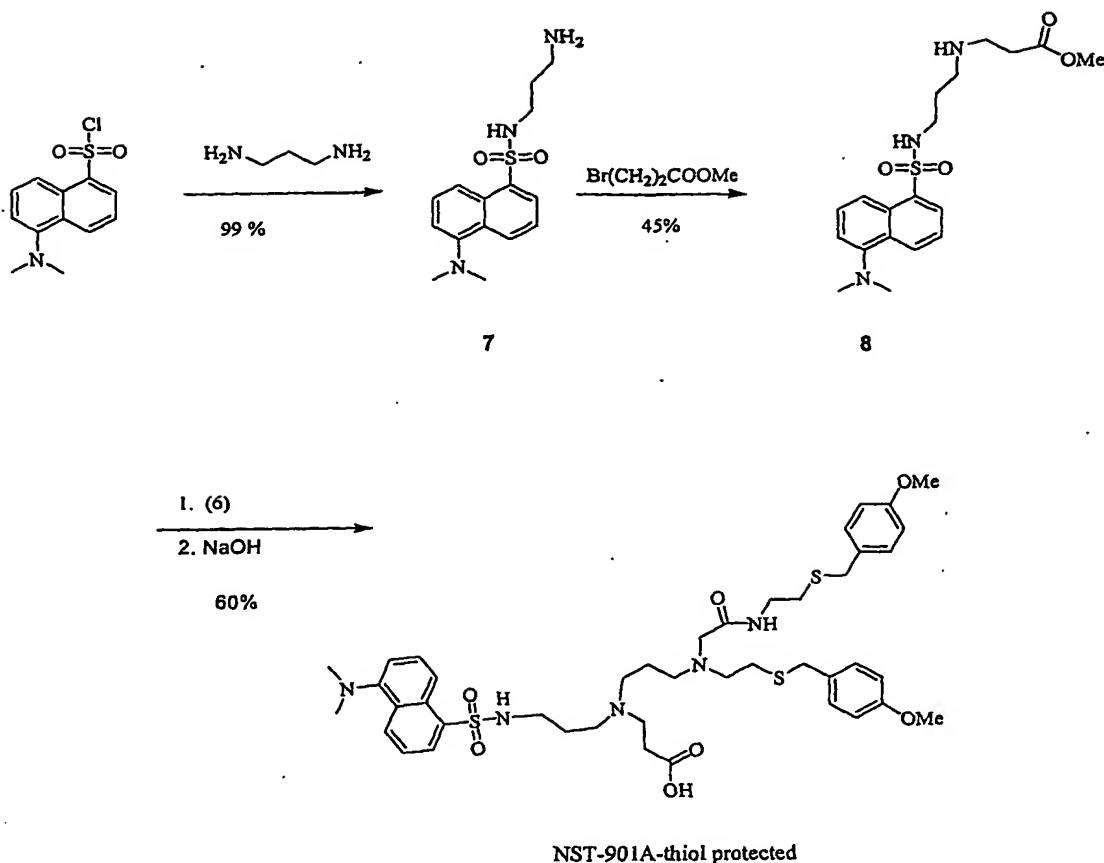
10 Dansyl chloride was treated in the cold with 1,3-diaminopropane (one equivalent) to provide 3-(5-dimethylamino-naphthalene-1-sulfonylamido) propylamine (7) in 99% yield. (7) (7.6g) was then treated with methyl-3-bromopropionate (4.2g) in refluxing acetonitrile overnight. After flash chromatography over silica, was obtained 3-(3-[5-dimethylamino-naphthalene-  
15 1-sulfonylamido] propionyl) amino propionic acid methyl ester (8) (4.4g).

Treatment of amine 8 (1.95g) with mesylate 6 (2.85g) in refluxing acetonitrile cleanly provided the coupled product in 60% yield after flash column chromatography over silica gel in >95% chemical purity (HPLC). Further hydrolysis of the methyl ester with methanolic 3N NaOH solution followed by  
20 acidification, afforded the corresponding thiol-protected NST-901A (2.5g, purity >95%).

m.p.: 53°C -55°C; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>), δ 8.43 (d, 1H, J = 8.43 Hz), 8.30 (dd, 1H, J = 5.92, 8.64 Hz), 8.1 (d, 1H, J = 7.26 Hz), 8.0 (bs, 1H), 7.80 (t, 1H, J = 4.5 Hz), 7.60 (dd, 2 H, J = 8.29, 7.36 Hz), 7.21 (d, 4H, J = 10.26 Hz),  
25 6.82 (d, 4 H, J = 10.39 Hz), 3.7 (s, 6H), 3.65(s, 4H), 3.42 (bq, 4H), 2.95 (s, 2H), 2.81 (s, 6H), 2.40 (m, 18H) 1.44 (bm, 4H). MS (ESI) 854.47 (M + H).

HPLC method: Hypersil BDS-C18 column; Solvent A H<sub>2</sub>O/0.1% TFA; Solvent B, 100%Acetonitrile; Linear Gradient of 40%B to 100% B over 17 min; Detection at 254 nm; *t*<sub>R</sub> = 7.606 min, 94% (area percent). TLC : *R*<sub>f</sub> 0.4 (Silica  
30 Whatman No. 4500-101, 95:5:1 Chloroform/Methanol/Acetic Acid).



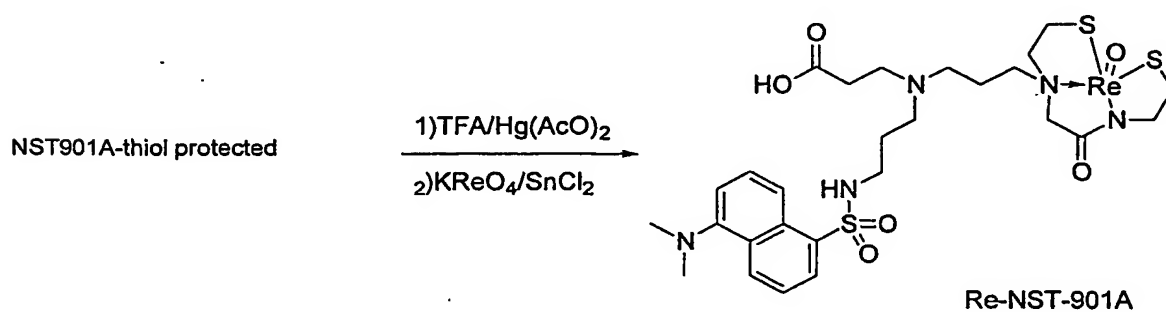


Scheme 1

5 **Example 2: Attachment of oxo-rhenium-NST901A complex (Re-NST901A),**  
**Scheme 2:**

50 mg of compound thiol-protected NST-901A were dissolved in 2 ml of trifluoro acetic acid (TFA) and anisole (50  $\mu$ l) was added, followed by 40 mg of mercuric acetate. The solution was kept at room temperature for one hour, with  
 10 occasional stirring. Then, TFA was evaporated; dry ether was added to the residue and the mixture was sonicated for 10 min. The yellowish powder was washed with ether and dried. The residue was dissolved in ethanol, a stream of hydrogen sulfide was bubbled through the solution for 10 min and the solution was agitated at room temperature for 1 hr. The black precipitate was filtered  
 15 through celite and the filtrate evaporated to dryness. The residue was re-dissolved in ethanol purged with N<sub>2</sub>. Twenty-two mg of potassium perhennate and 17 mg

of stannous chloride dissolved both in deoxygenated 0.05M HCl were then added to the boiling solution. The solution was boiled overnight, filtered through celite and the solvent was evaporated. The residue was applied to a silica gel column and eluted with a 0-5% gradient of methanol in dichloromethane (DCM).  
5 Brown-purple colored chelate was eluted (3% MeOH). This fraction exhibited fluorescence when irradiated at a wavelength of 360 nm, and was found to be over 95% pure (TLC). Evaporation leaves 18 mg of desired Re-NST901A.



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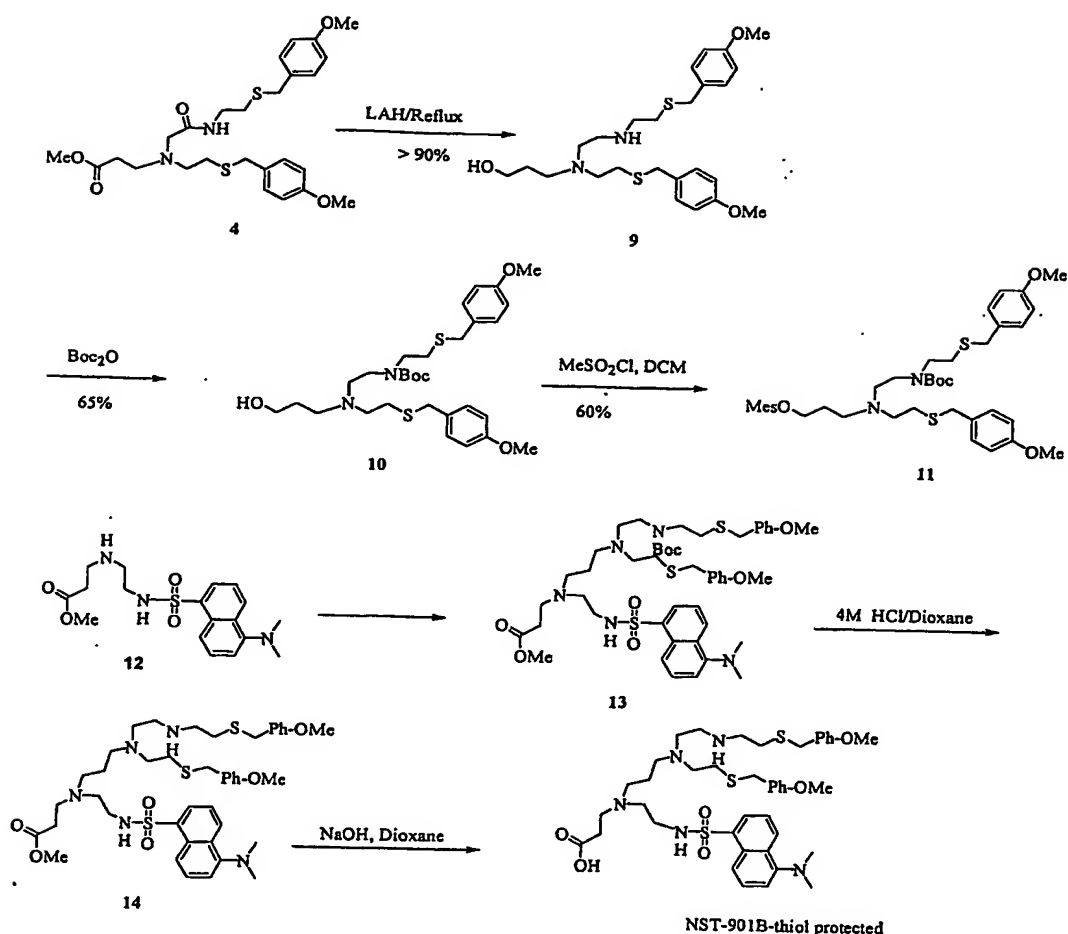
Scheme 2

**Example 3: Synthesis of thiol-protected NST901B (Scheme 3):**

Compound 4 of Scheme 1 (5.6g) was reduced with lithium aluminum hydride in refluxing tetrahydrofuran (THF) overnight. Common workup afforded amino alcohol 9 in high yields. Di-*tert*-butyl dicarbonate in acetonitril and catalytic amounts of dimethyl amino pyridine were used to protect the amino group. The regular workup provided a thick oil. Compound 10 was then purified by silica gel chromatography with *isopropyl* alcohol/dichloromethane as solvent in 65%. 10 was further reacted, in dichloromethane, with methanesulfonyl chloride in the presence of triethylamine. Workup and purification with silica gel afforded 11.

Mesylate 11 (0.8g) was coupled with 3-[2-(5-dimethylamino-naphthlene-1-sulfonamino)-ethylamino]-propionic acid methyl ester 12 (prepared similar to 7 in scheme 1, diamino ethane replacing 1,3, diamino propane) in acetonitrile at 45 °C, resulting in approximately 57% of the desired product 13 (0.6 g). The butyl oxycabonyl (boc) protecting group was removed by treatment of a solution of 13 (0.6 g) in methanol (10 mL), with a 2 M solution of hydrochloric acid in dioxane overnight, to provide 14 (0.52 g) in almost quantitative yield, with a chemical purity of >95% [assayed by HPLC analysis using a C18 Hypersil BDS column (*t<sub>r</sub>*, 8.718 min, 30% ACN/H<sub>2</sub>O to 100% ACN linear gradient over 13 min)]. Final hydrolysis of 14 (0.52 g) afforded the desired compound thiol-protected NST 901B.

m.p. 126-130 °C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 9.76 (bs, 2H), 8.56 (bs, 2H), 8.35 (d, *J* = 8.3 Hz, 1H), 8.18 (d, 6.80 Hz, 1H), 7.68 (dd, *J* = 8.56, *J* = 9.01 Hz, 2H), 7.41 (bs, 1H), 7.29 (d, *J* = 13.3 Hz, 4H), 6.87 (d, *J* = 13.31 Hz, 4 H), 3.73 (s, 8H), 3.56 (s, 6H), 3.4-3.32 (bm, 16H), 2.99 (s, 6 H). 2.76 (m, 4H), 2.25 (m, 2H). MS (ESI) 826 (M + H).

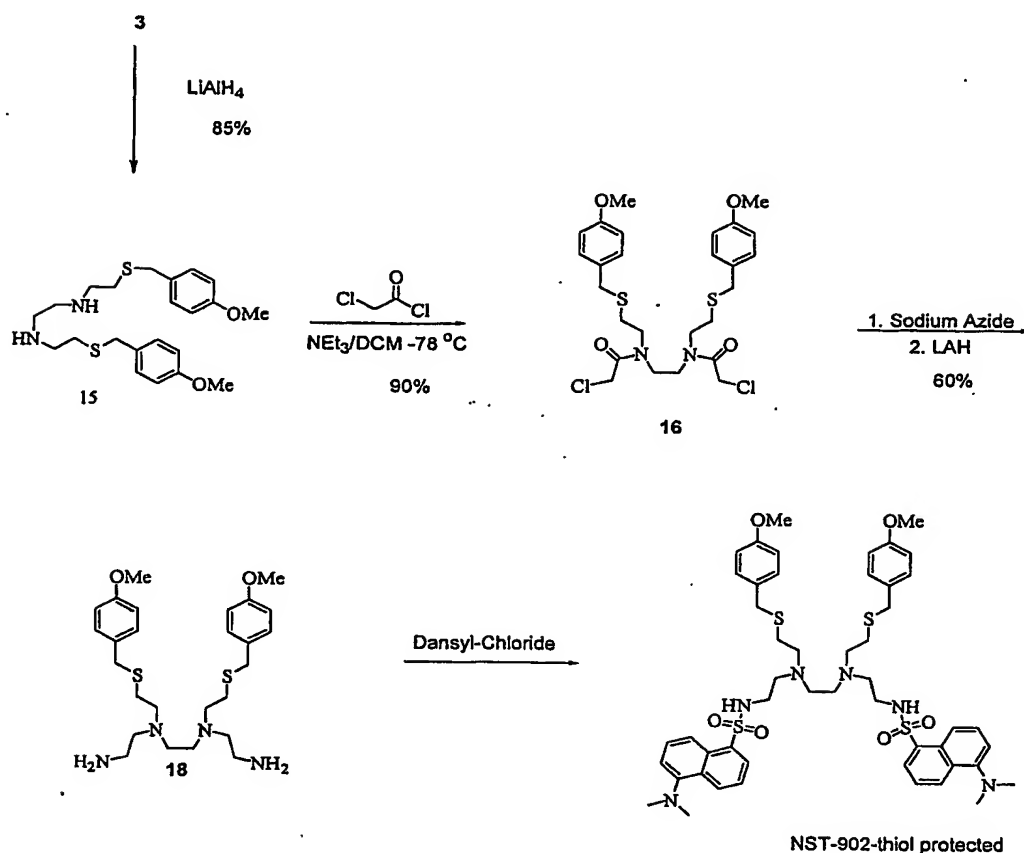


Scheme 3

#### Example 4: Synthesis of thiol-protected NST902 (Scheme 4):

Compound 3 of Scheme 1 was reduced with lithium aluminum hydride (LAH) to yield 15 in 85%. 2g of 15 were reacted with 2 equivalents of chloroacetyl chloride in DCM in the presence of triethyl amine at  $-78^\circ\text{C}$  for 1hr to yield 2.75g of 16. 1g of 16 was taken up in dimethylformamide (DMF, 10ml) and treated with 5 equivalents of sodium azide at  $50^\circ\text{C}$  overnight. After a usual workup, the reaction was concentrated to dryness to provide the bis-azide adduct 17 in 85% yield with a chemical purity of 95% (area percent) by HPLC analysis. The resulting oil was then treated at reflux with 1M LAH solution. After a workup, 0.5 g of the diamine 18 was isolated in good purity. Coupling of the dansyl chloride was performed in DCM to provide oil which was then purified by

flash column chromatography to provide 0.5 g of the target molecule



5

Scheme 4

**Example 5: Selective binding of Re-NST901A to apoptotic cells; fluorescent microscopy:**

HeLa S3 cells (ATCC CCL-2.2) were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 2mM of L-glutamine; 100 units/ml of penicillin; 100 µg/ml of streptomycin; 12.5 units/ml of nystatin and 10 % of fetal calf serum (FCS). Cells were seeded at a density of 5x10<sup>6</sup> cells / plate, on a 10 cm<sup>3</sup> culture plates, in a volume of 10 ml, and were allowed to age by incubating the culture for 96 hours without exchange of the growth medium. As a result, a marked percentage of the cells became apoptotic. Cells were harvested using a

cell scraper, separated to single cells by passage through a syringe with a 18G needle, and re-suspended at a density of  $10^6$  cells/ml in PBS buffer at pH=7.4.

Binding of the Re-NST901A was measured by detection of the intensity of its inherent fluorescence upon membrane binding (excitation wavelength of 360nm, emission at 530nm). For said measurement of binding, cells were washed twice with TBS (Tris buffered saline; 10 mM Tris, pH 7.4, 150 mM NaCl). Re-NST901A was suspended in 0.1M sodium carbonate buffer at pH 10.5, at a concentration of 10mM. Cells were incubated with Re-NST901A for 10 minutes at a final concentration of 250 $\mu$ M in a total volume of 100 $\mu$ l, and were then taken for fluorescent microscopy. Observations were performed with an Olympus fluorescent microscope (model IX70).

Strong and selective uptake of Re-NST901A by the apoptotic cells was observed, and the compound manifested marked accumulation within these cells. By contrast, viable, intact cells did not bind / accumulate the compound (Fig. 1, magnification X400).

**Example 6: Detection of chemotherapy-induced apoptosis of mouse small intestine epithelial cells *in vivo* by Re-NST901A:**

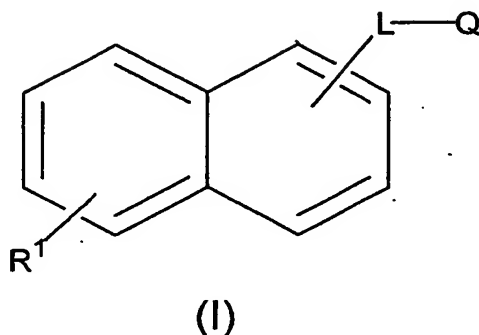
Gastrointestinal damage is often observed during administration of anti-cancer therapy. In particular, the small intestine crypts manifest apoptosis of epithelial cells as an early response to chemotherapy and irradiation (Keefe, D.M.K., *et al.*, *Gut*, 47:632-637, 2000). Detection of chemotherapy-induced, small intestine epithelial apoptosis by Re-NST901A *in vivo* was therefore examined.

Twelve-week old Balb/c mice were treated intravenously with a single dose of a combination of Taxol (27 mg/kg) and cyclophosphamide (300 mg/kg). After 24 hours, all animals were injected intravenously with 210 mg/kg of Re-NST901A. Two hours later, animals were sacrificed, the small intestine was removed and and cryo-sections were prepared for fluorescent microscopy.

Multiple apoptotic cells could be detected using H/E staining in the small intestinal crypts of the chemotherapy-treated mice (Fig. 2A). Said apoptotic cells manifested eosinophilic cytoplasm, and condensed and fragmented nuclei. Strong, selective uptake of Re-NST901A by the apoptotic cells was detected in  
5 the small intestinal crypts of the chemotherapy-treated mice. By contrast, no significant uptake of Re-NST901A was observed in non-apoptotic cells in the crypts (Fig. 2B). This exemplifies the potential usefulness of the compounds of the invention as a tool for an early and sensitive monitoring of this adverse effect of chemotherapy, allowing its detection even after a single dose of anti-cancer  
10 treatment.

CLAIMS:

1. A compound of the formula  $E_i$ , wherein  $i$  is 1 or 2 and  $E$  is a group having the formula (I):



5

including pharmaceutically acceptable salts, metal chelates and hydrates of the compound of formula (I); wherein

Q is a marker for imaging or a metal chelator, said marker for imaging being  
10 selected from the group comprising of a fluorescent label, a radio-label, a marker for X-ray, a marker for MRI, a marker for PET scan, or a label capable of undergoing an enzymatic reaction that produces a detectable color;

L is a linking group having the formula  $D-U-B-Y^1-A-Y^2$ , wherein D is selected from the group consisting of O, S, SO, SO<sub>2</sub>, SO<sub>2</sub>NH, NHSO<sub>2</sub>, NH, PO,  
15 PO<sub>2</sub>, PO(NH)<sub>2</sub>, NHPO<sub>2</sub>, CO, CO<sub>2</sub>, NHCO, CONH, SO<sub>2</sub>NHCHCO<sub>2</sub>, and SO<sub>2</sub>NHCO;

U is selected from optionally substituted C<sub>1</sub>-C<sub>6</sub> alkylene, C<sub>2</sub>-C<sub>6</sub> alkenylene, C<sub>3</sub>-C<sub>6</sub> branched alkylene, and C<sub>3</sub>-C<sub>6</sub> branched alkenylene;

Y<sup>1</sup> and Y<sup>2</sup> may be the same or different, and are each independently selected  
20 from null and U;

B is null or is selected from O, S, NH, C=O, CH<sub>2</sub>-OH, (CH<sub>2</sub>)<sub>2</sub>-OH, CH<sub>2</sub>-NH<sub>2</sub>, (CH<sub>2</sub>)<sub>2</sub>-NH<sub>2</sub>, aryl, heteroaryl, and combinations thereof;

A groups may be the same or different, and are charged moieties at pH of about 7; said charged moieties being either positively-charged, negatively-charged or in zwitterion form;

$R^1$  is  $WR_b^2$ , where W is null or is selected from N, O, S and C;

5  $R^2$  represents hydrogen or a  $C_1$ - $C_6$  alkyl;  $R^2$  moieties may be either the same or different; and

b is 1, 2 or 3;

where when  $i = 2$ , the compound of formula (I) is in dimeric form (E-E), said dimeric form having:

10 a single Q moiety;  
a single A group; and

in the case that Q is a charged group at pH of about 7, said A group of said E-E dimeric form being as defined above or null.

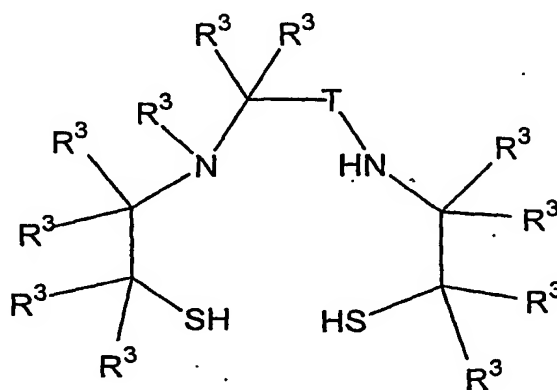
2. The compound of Claim 1, wherein Q is a metal chelator.

15 3. The compound of Claim 1, wherein Q is a marker for imaging selected from the group comprising of a fluorescent label, a radio-label, a marker for X-ray, a marker for MRI, a marker for PET scan, or a label capable of undergoing an enzymatic reaction that produces a detectable color.

4. The compound of anyone of Claims 1-3, wherein U is an optionally  
20 substituted  $C_1$ - $C_5$  alkylene group;  $R^1$  is  $NR^2_2$ , wherein each  $R^2$  group is selected independently from hydrogen and  $C_1$ - $C_4$  alkyl; A is a charged group at pH of about 7, said charged group being either positively-charged, negatively-charged or in zwitterion form, formed from at least one amino group and at least one acidic group, the acidic group being selected from carboxylic, phosphoric, phosphatic,  
25 sulfonic and sulfuric acid; where the amino group may also be a quaternary ammonium ion.

5. The compound of Claim 1 wherein the B group is other than null and the D-U moiety has the formula  $-SO_2NH-(CH_2)_n$ , wherein n is an integer between 1-3 and Q, A and Y are as defined in Claim 1.

6. The compound of Claim 1, wherein B is null, A is a positively-charged group at a pH of about 7,  $Y^1$  is null,  $Y^2$  and Q are as defined in Claim 1, and D-U is of the formula  $-SO_2NH-(CH_2)_n$ , wherein n is an integer, independently selected from 1-3.
7. The compound of anyone of Claims 2 to 4, where Q forms metal chelates with Technetium, Gallium or Rhenium isotopes.
8. The compound of Claim 2, where the chelation of Q to a metal is accomplished through a combination of nitrogen, sulfur and/or oxygen atoms comprised by Q.
9. The compound of Claim 8, wherein the chelation of Q to a metal is accomplished through a combination of three nitrogen atoms and a sulfur atom, two nitrogen atoms and two sulfur atoms or a nitrogen atom and three sulfur atoms.
10. The compound of Claim 2 or 4, wherein Q is selected from diaminedithiols, monoamine-monoamide-bisthiols (MAMA), triamide-monothiols, and monoamine-diamide-monothiols.
11. The compound of Claim 9, wherein Q is a metal chelator of the following formula (II):

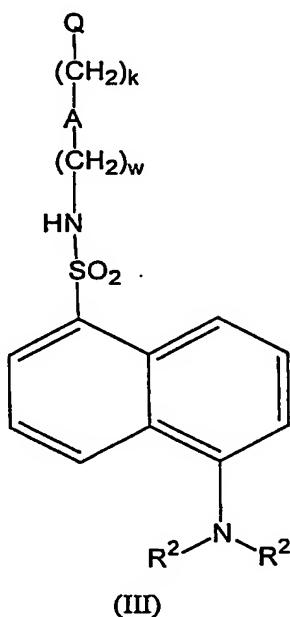


(II)

wherein T is  $CH_2$  or  $C=O$ ;  $R^3$  groups may be the same or different, and are each independently selected from hydrogen, methyl, ethyl,  $COOH$  and  $CH_2COOH$ ;

wherein the number of  $R^3$  moieties comprising a carboxyl group  $\leq 2$ ; including pharmaceutically acceptable salts, and hydrates thereof; said metal chelator Q being bound to L in the compound of formula (I) via an  $R^3$  group or a nitrogen atom of the chelator.

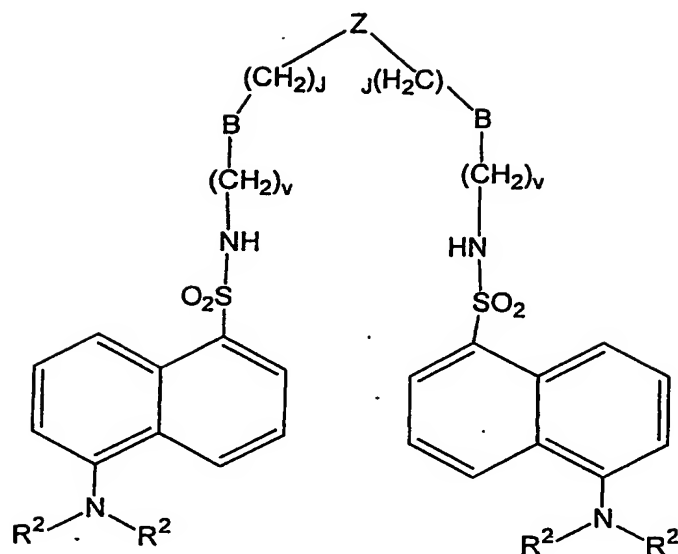
- 5 12. The compound of Claim 1 having the following formula (III):



including pharmaceutically acceptable salts, hydrates and metal chelates of the  
10 compound of the formula III;

wherein A and Q have the same meanings as defined in Claim 1; and  $R^2$  groups may be the same or different and are each independently selected from hydrogen and  $C_1$ - $C_4$  alkyl and k and w are integers selected from 1-3.

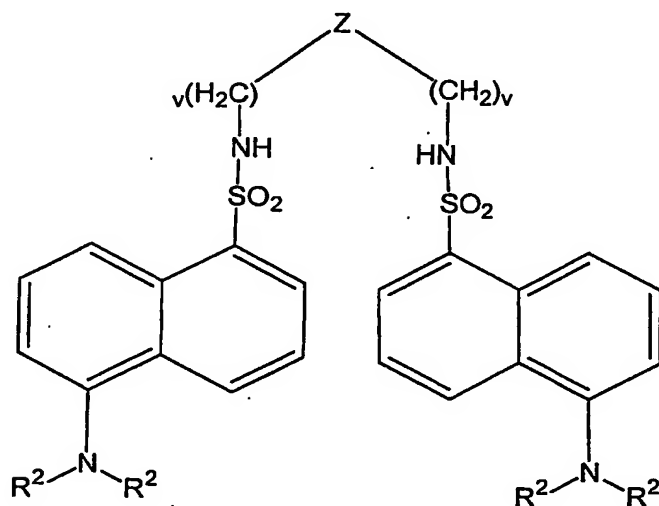
13. The compound of Claim 1 having the following formula (IV):



(IV)

wherein  $R^2$  has the same meanings as defined in Claim 1; Z is Q or A- $Y^2$ -Q where Q, A and  $Y^2$  are as defined in Claim 1; and v and j are each an integer selected from 0-3; including pharmaceutically acceptable salts, hydrates and metal chelates  
5 of the compound of the formula IV.

14. The compound of Claim 1 having the following formula (V):

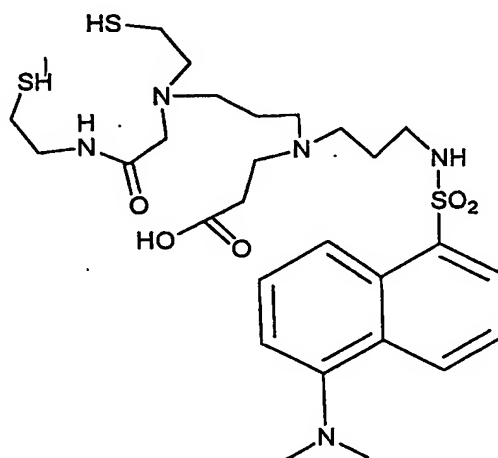


(V)

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of the formula V;

wherein  $R^2$  has the same meanings as defined in Claim 1; Z is selected from Q,  $A-Y^2-Q$ ,  $B-Y^1-A-Q$ , and  $B-Y^1-Q$  where Q, A,  $Y^1$ ,  $Y^2$  and B are as defined in Claim 1; and v is an integer of 1-6; wherein in the case that Z is  $B-Y^1-Q$ , Q is a charged group at pH of about 7.

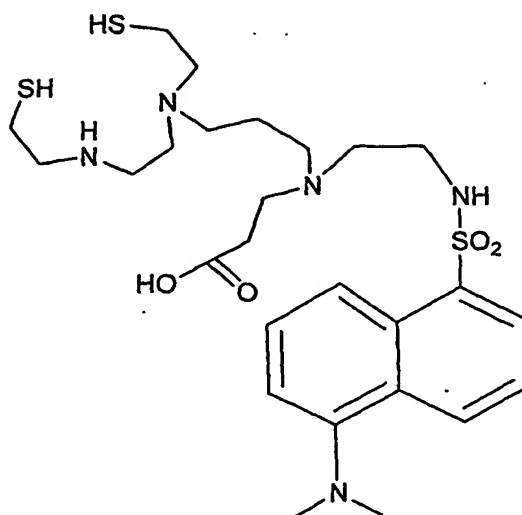
15. The compound of Claim 1 having the following formula VI:



(VI)

10 including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula (VI)

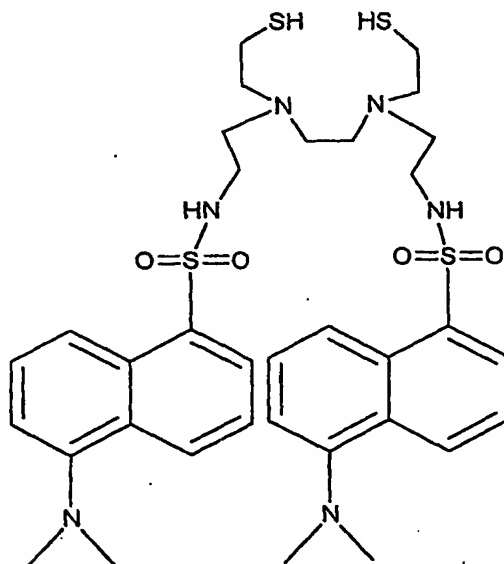
16. The compound of Claim 1 having the following formula VII:



(VII)

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula (VII).

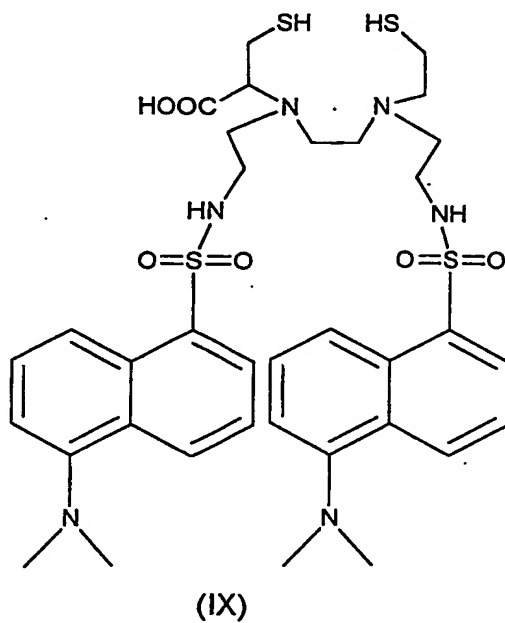
- 5 17. The compound of Claim 1 having the following formula VIII:



(VIII)

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula (VIII).

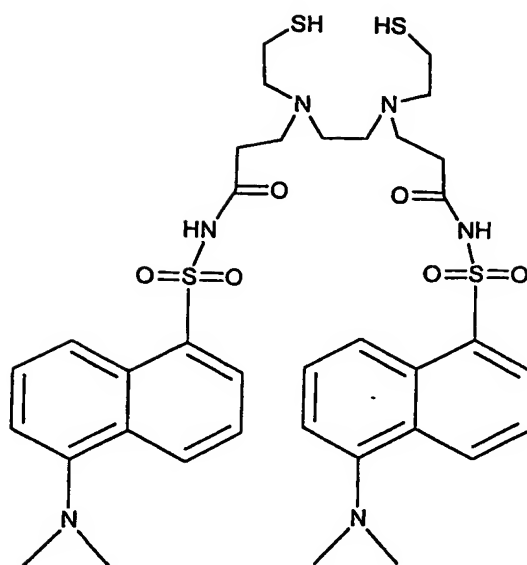
18. The compound of Claim 1 having the following formula IX:



5

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula (IX).

19. The compound of Claim 1 having the following formula X:



(X)

including pharmaceutically acceptable salts, hydrates and metal chelates of the compound of formula (X)

20. The compound of any one of Claims 15-19 wherein the chelated metal is selected among Technetium and Rhenium radioisotopes.

21. The compound of Claim 1 for use in the diagnosis of medical disorders in which cells undergo PNOM.

22. A diagnostic agent comprising a compound of the formula  $E_i$  as defined in Claim 1 and a metal, said metal being chelated by said compound of the formula  $E_i$  through a Q moiety of  $E_i$ .

23. A diagnostic agent being a compound of the formula  $E_i$  as defined in Claim 1, wherein Q is a radioisotope covalently linked to the L moiety.

24. A diagnostic agent being a compound of the formula  $E_i$  as defined in Claim 1, having fluorescence properties.

25. A diagnostic kit comprising one or more vials containing a sterile formulation comprised of a predetermined amount of a diagnostic agent according to anyone of Claims 22-24 and optionally other components, such as stabilization aids, solubilization aids or bacteriostats.

26. A diagnostic kit according to Claim 25, wherein the one or more vials that contain all or part of the formulation can independently be in the form of a sterile solution or a lyophilized solid.

27. A diagnostic composition for the detection of a perturbed membrane in a sample of biological cells, *in vitro*, *ex vivo*, *in vivo* or for clinical imaging, comprising as an active component a compound of the formula E<sub>i</sub> as defined in Claim 1, together with a biologically acceptable carrier, said active component having detectable properties of its own, being capable of chelating a detectable label or being chelated to a detectable label.

28. The diagnostic composition of Claim 27, wherein said detectable label is a metal.

29. The diagnostic composition of Claim 27, wherein the active compound has detectable properties in its own, being detected by fluorescent microscope, or by flow cytometric equipment.

30. The diagnostic composition of Claim 27, wherein the active compound has detectable properties in its own, being detected by radioimaging techniques.

31. The diagnostic composition of Claim 28, being a diagnostic radiocomposition for radioimaging, wherein the active compound is in the form of a metal chelate, and said metal is a radioisotope.

32. The diagnostic composition of Claim 31, for use in single photon emission computed tomography (SPECT), wherein the metal is a radioisotope of a metal selected from Tc, In, Cu, Ga, Xe, Tl and Re.

33. The diagnostic composition of Claim 32 wherein the metal is a radioisotope of a metal selected from Tc and Re.

34. The diagnostic radiocomposition of Claim 33, wherein the active compound is radiolabeled with <sup>99m</sup>Tc.

35. The diagnostic composition of Claim 27, being a diagnostic radiocomposition for radioimaging, wherein the radiolabel is a covalently linked radioisotope.

36. The diagnostic composition of Claim 35, being a diagnostic radiocomposition for single photon emission computed tomography (SPECT).
37. The diagnostic composition of Claim 36, wherein the radiolabel is a radioisotope of iodine.
- 5 38. The diagnostic composition of Claim 27, being a diagnostic radiocomposition for positron emission tomography (PET), wherein the radiolabel is a covalently linked radioisotope.
39. The diagnostic composition of Claim 38, wherein the radioisotope is selected from  $^{18}\text{F}$ ,  $^{15}\text{O}$ ,  $^{18}\text{O}$ ,  $^{11}\text{C}$ ,  $^{13}\text{C}$ ,  $^{124}\text{I}$ ,  $^{13}\text{N}$  and  $^{75}\text{Br}$ .
- 10 40. The diagnostic composition of Claim 27 being a MRI contrast composition.
41. The diagnostic composition of Claim 40, wherein the active compound is in the form of a metal chelate, the metal being a paramagnetic metal ion.
42. The diagnostic composition of Claim 27 being a X-ray or computerized tomography (CT) contrast composition.
- 15 43. The agent of anyone of Claims 22-24 for use in the diagnosis of medical disorders in which cells undergo PNOM.
44. The agent of anyone of Claims 22-24 for the detection of cells undergoing a death process.
45. The agent of Claim 44 for the detection of cells undergoing apoptosis.
- 20 46. The agent of anyone of Claims 22-24 for the detection of procoagulant particles, selected among activated platelets, platelet-derived microparticles, and apoptotic bodies.
47. The agent of anyone of Claims 22- 24 for the detection of a blood clot.
48. The agent of Claims 22- 24 for the detection of activated inflammatory cells,
- 25 selected among activated white blood cells and activated tissue macrophages.
49. The agent of Claim 43, for detecting the presence of a disease condition in a person already known to have the disease, for the purpose of evaluating the disease severity, monitoring disease progression, and/or monitoring a response to therapeutic modalities.

50. The agent of Claim 43, for the detection and diagnosis of a disease selected from: diseases characterized by occurrence of excessive apoptosis, degenerative disorders, neurodegenerative disorders, Parkinson's disease, Alzheimer's disease, Huntington chorea, AIDS, myelodysplastic syndromes, ischemic or toxic insults,  
5 graft cell loss during transplant rejection;

diseases manifested by excessive blood clotting; arterial or venous thrombosis, thrombo-embolism, myocardial infarction, cerebral stroke, deep vein thrombosis, disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), sickle cell diseases, thalassemia,  
10 antiphospholipid antibody syndrome, systemic lupus erythematosus;

inflammatory disorders, and / or diseases associated with immune-mediated etiology or pathogenesis; auto-immune disorders, antiphospholipid antibody syndrome, systemic lupus erythematosus, connective tissue disorders such as rheumatoid arthritis, scleroderma; thyroiditis; dermatological disorders, pemphigus,  
15 erythema nodosum; autoimmune hematological disorders; autoimmune neurological disorders, myasthenia gravis; multiple sclerosis; inflammatory bowel disorders, ulcerative colitis; vasculitis.

51. The agent of Claim 49, wherein said detection is used to monitor adverse effects of anti-cancer treatments.

20 52. The agent of Claim 49, wherein said detection is used to monitor death of tumor cells in response to anti-cancer treatment, selected among chemotherapy and radiotherapy.

53. The agent of Claim 49, wherein said detection is used to characterize the intrinsic apoptotic load within a tumor, the level of aggressiveness of a  
25 tumor, or to detect metastases.

54. The agent of Claim 49, wherein said detection is used to monitor graft survival after organ transplantation.

55. The agent of Claim 49, wherein said detection is used for diagnosis of atherosclerotic plaques.

56. The agent of Claim 55, wherein said detection is used for diagnosis of unstable atherosclerotic plaques.
57. The agent of Claim 49, wherein said detection is used in the monitoring of response to cytoprotective therapy in a disease characterized  
5 by excessive apoptosis, said response being inhibition of cell death.
58. A diagnostic kit comprising one or more vials containing a sterile formulation comprised of a predetermined amount of a diagnostic composition according to Claim 27 and optionally other components, such as stabilization aids, solubilization aids or bacteriostats.
- 10 59. A diagnostic kit according to Claim 58, wherein the one or more vials that contain all or part of the formulation can independently be in the form of a sterile solution or a lyophilized solid.
60. A method for the detection of cells having perturbed membranes (PM cells) in a cell sample, the method comprising:
- 15 (i) contacting the cell sample with a diagnostic agent according to anyone of Claims 22-24 under conditions enabling binding of said agent to biological membranes; and
- (ii) detecting bound agent to said cells; the presence of a significant amount of bound agent indicating the presence of PM in said cells.
- 20 61. A method for the detection of physiological disorders characterized by the presence of cells having perturbed membranes (PM cells), and/or medical disorders in which PM cells have an etiological or a pathogenetic role, such method comprising:
- (1) administering a diagnostic composition according to anyone of Claims  
25 27-42 to a patient; and
- (2) imaging the patient using an appropriate imaging technique.
62. A method according to Claim 61 wherein the diagnostic composition comprises a radiolabel, and the detection of the medical disorders is by a radioimaging technique.

63. A method according to Claim 61 wherein the diagnostic composition comprises a radiolabel, and the detection of the medical disorders is by single photon emission computed tomography (SPECT).

5 64. A method according to Claim 61 wherein the diagnostic composition comprises a radiolabel, and the detection of the medical disorders is by positron emission tomography (PET).

65. A method according to Claim 61 wherein the diagnostic composition is an X-ray contrast agent.

66. A method according to Claim 61 wherein the diagnostic composition  
10 comprises a magnetic resonance imaging (MRI) contrast agent.

67. A method according to Claim 61 wherein the diagnostic composition comprises a fluorescent label.

68. A method according to Claim 61 or 60, for the detection of cells undergoing a death process.

15 69. A method according to Claim 68, for the detection of cells undergoing apoptosis.

70. A method according to Claims 61 or 60, for the detection of procoagulant particles, selected among activated platelets, platelet-derived microparticles, and apoptotic bodies.

20 71. A method according to Claim 61 or 60 for the detection of a blood clot.

72. A method according to Claim 61 or 60, for the detection of activated inflammatory cells, selected among activated white blood cells and activated tissue macrophages.

73. A method according to Claim 61, for the diagnosis of a disease selected  
25 from: diseases characterized by occurrence of excessive apoptosis; degenerative disorders, neurodegenerative disorders, Parkinson's disease, Alzheimer's disease, Huntington chorea, AIDS, myelodysplastic syndromes, ischemic or toxic insults, graft cell loss during transplant rejection;

diseases manifested by excessive blood clotting; arterial or venous  
30 thrombosis, thrombo-embolism, myocardial infarction, cerebral stroke, deep vein

thrombosis, disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), sickle cell diseases, thalassemia, antiphospholipid antibody syndrome, systemic lupus erythematosus; inflammatory disorders, and / or diseases associated with immune-mediated etiology or pathogenesis; auto-immune disorders, antiphospholipid antibody syndrome, systemic lupus erythematosus, connective tissue disorders such as rheumatoid arthritis, scleroderma; thyroiditis; dermatological disorders, pemphigus, erythema nodosum; autoimmune hematological disorders; autoimmune neurological disorders, myasthenia gravis; multiple sclerosis; inflammatory bowel disorders, ulcerative colitis; vasculitis.

74. A method according to Claim 61, for the detection of atherosclerotic plaques.

75. A method according to Claim 74, for the detection of unstable atherosclerotic plaques.

76. A method according to Claim 61, for detection of cell death within a tumor, for monitoring of aggressiveness of a tumor, or for detection of metastases of a tumor.

77. A method according to Claim 61, for monitoring death of tumor cells in response to an anti-cancer treatment, selected among chemotherapy and radiotherapy.

78. A method according to Claim 61, for monitoring adverse effects of an anti-cancer treatment, wherein said adverse effects being death of normal cells.

79. A method according to Claim 61, for monitoring of survival of a grafted organ after transplantation.

80. A method according to Claim 61, for monitoring of response to cytoprotective therapy in a disease characterized by excessive apoptosis, said response being inhibition of cell death.

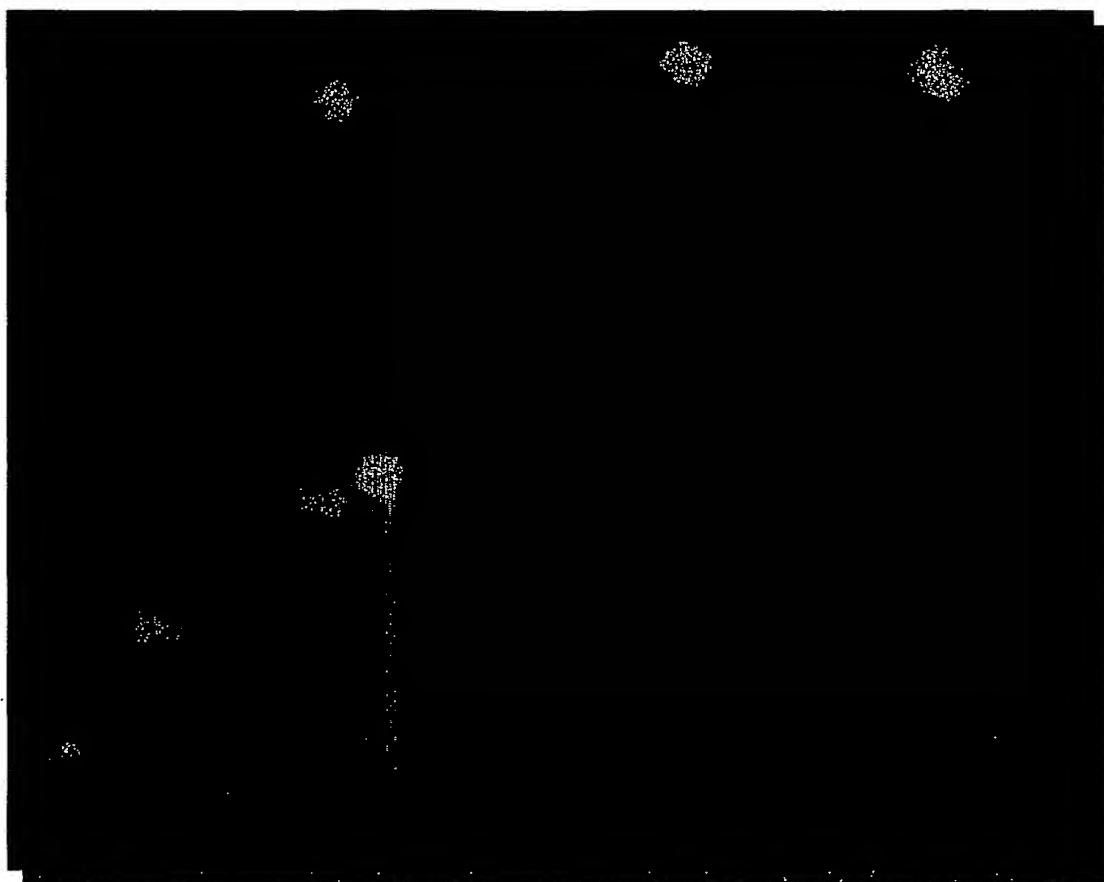
For the Applicants  
REINHOLD COHN AND PARTNERS

By

*Yigal Fraum*

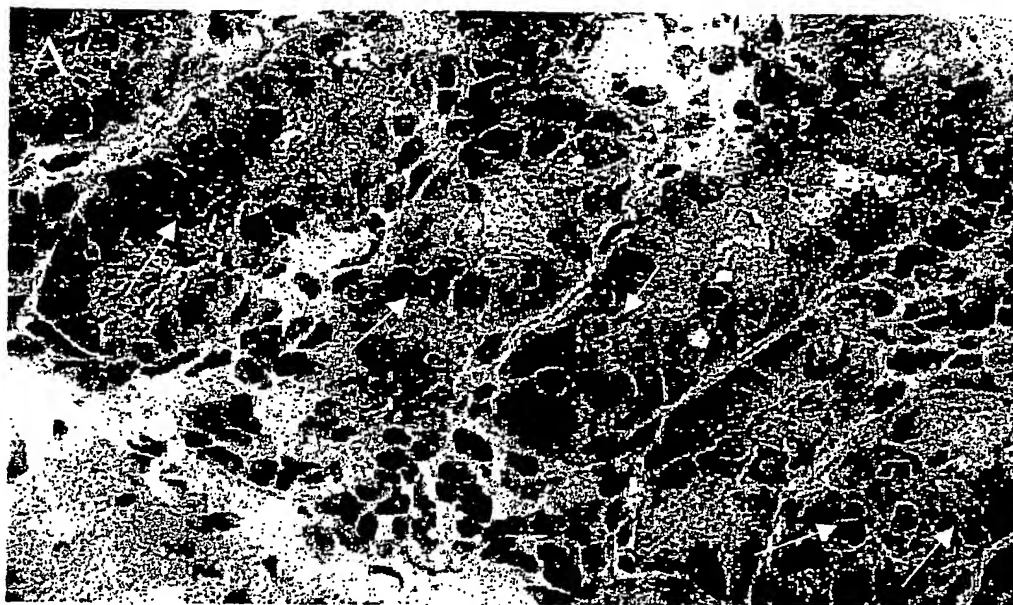
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**Figure 1**



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**Figure 2**



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